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Live weight gain and urinary nitrogen excretion of dairy heifers grazing pasture, chicory and plantain

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A Dissertation

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Hazel Carr

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science with Honours.

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The objective of this study was to determine the effect of feeding plantain and chicory at different proportions in the diet with perennial ryegrass-white clover pasture on the live weight (LW) gain, urinary nitrogen (N) excretion, and urination behaviour of dairy heifers grazing in autumn. The study was completed at Lincoln University's Ashley Dene Pastoral Systems Research Farm, Canterbury, New Zealand and had a duration of 32 d in autumn. A total of 60 Friesian x Jersey dairy heifers aged 8-9 months, were blocked into five dietary treatments balanced for their LW and breeding worth; 100% perennial ryegrass-white clover pasture (R; n = 12); 50% pasture + 50% chicory (50%C; n = 10); 75% pasture + 25% chicory (25%C; n=12); 50% pasture + 50% plantain (50%P; n = 12); and 75% pasture + 25% plantain (25%P; n=12). A fresh allocation of the herbage was offered every 3 d with herbage allowance calculated according to feed requirement for maintenance plus gain of 1.0 kg LW/d. The results showed no significant difference in LW gain between treatments (mean = 0.70 kg/d/hf; P = 0.11). There was no significant difference in urine N, NH_3, BUN, glucose, urea, faecal DM and microbial protein synthesis between all treatments (P>0.05). Allantoin was significantly lower in R and 25%P (2.45mmol/L and 2.43mmol/L respectively) and higher in 25%C (4.17mmol/L) (P=0.029). Uric acid was significantly lower in R and 25%P (0.17mmol/L and 0.16mmolL respectively) and higher in 25%C (0.28mmol/L) (P =0.033). A small difference was apparent in creatinine, where R and 25%P were lower (0.75mmol/L and 0.78mmo/L) and 25%C was higher (1.18mmol/L) (P=0.07). A significantly higher urination frequency was observed in R and a significantly lower urination frequency in 50%C (5.91 urination/hf/6h and 2.33 urinations/hf/6h respectively; p-value <0.001) than 25%C, 50%P and 25%P (mean = 3.92 urination/hf/6h). 25%C & 25%P both had smaller urine patch areas when compared to other treatments (P=0.071) therefore indicating a lower volume per urination event. Data from this study indicates that there is a benefit to feeding chicory and plantain at 50% and 25% of pasture area on N loss to the environment through urination frequency and volume and that feeding chicory and plantain at these percentages has no negative effect on LW gain.

Keywords: microbial protein, plasma urea nitrogen, sustainability, urinary N excretion,

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Chapter 1

Introduction

Livestock production systems in New Zealand (NZ) are primarily based on animal grazing pasture containing perennial ryegrass (*Loliun perenne L.*) and white clover (*Trifolium repens L.*), normally grown in a simple binary mixture. While this mixture has many desirable characteristics (Kemp *et al*., 2010), it may suffer from low and variable herbage growth in summer and autumn, and poor quality in spring and summer (Valentine and Kemp, 2007; Parsons *et al.,* 2011). These pasture factors may limit nutrient intake by grazing ruminants, and constrain livestock production and reproductive performance (Burke *et al*., 2002; Muir *et al*., 2014). In recent years, there has been increased interest in the use of deep-rooting forage herbs, such as chicory (*Cichorus intybus L.*) and plantain (*Plantago lanceolata L.*), to sustain pasture quality and production throughout the spring to autumn period, and enhance animal nutrient intake and performance (Schreurs *et al*. 2002; Chapman *et al*. 2008; Muir *et al*., 2014).

Previous studies show sheep, beef and deer grazing on swards containing plantain and chicory had higher gain of live weight (LW) than animals grazing on perennial ryegrass-white clover pasture. The higher LW gain has been assigned to a range of factors, including lowered internal parasite load (Scales, 1995; Barry, 1998; Parish *et al*., 2012) and greater dry matter intake (DMI) (Schreurs *et al*., 2002; Golding *et al*., 2008; Kenyon *et al*., 2010). Similarly, lactating ewes (Hutton *et al*., 2011) and dairy cows (Waghorn and Clark, 2004) grazing swards containing chicory and plantain produced more milk than those offered perennial ryegrass-white clover pasture. However, there have been few studies into the effects of feeding chicory and plantain on dairy heifer LW gain. This is despite achievement of LW targets at critical growth stages (e.g. mating and calving) being crucial to ensure heifer reproductive performance and milk production (Macdonald *et al.,* 2005). A recent report suggested that up to 70% of the rising two-year old dairy heifers in NZ failed to achieve LW target at mating (McNaughton and Lopdell, 2012), and this was partly attributed by poor quality pasture and low DMI.

Grazing perennial ryegrass-white clover pasture often provides an excessive amount of nitrogen (**N**) relative to livestock requirements (Pacheco and Waghorn, 2008); in turn, the excretion of surplus N through urination can lead to environmental pollution through greenhouse gas emissions (e.g. nitrous oxide) and nitrate leaching to ground water (Di and Cameron, 2002). Recent studies show pastures containing chicory and plantain may potentially offer a strategy to reduce the environmental impact of livestock farming (Pembleton *et al*., 2015). For example, lactating dairy cows grazing a pasture mixture containing 36% chicory and 18% plantain, with perennial ryegrass had lower urinary N excretion (**UN**) and urinary N concentration than cows grazed perennial ryegrass-white clover pasture (Totty *et al*., 2013). Further, Deaker *et al*. (1994) and Barry (1998) showed sheep had lower serum urea and rumen ammonia concentrations when fed with plantain and chicory, compared to when they were offered with pasture. However a limited number of studies have investigated which herb species and how much of each species is needed to achieve a reduction in UN from livestock, without losing productivity. Further there are no known studies of the effect of chicory and plantain on dairy heifers. Therefore, the objective of this study was to determine the effect of the proportion of plantain and chicory in the diet with pasture on the LW gain, urinary nitrogen (N) excretion, and grazing behaviour of dairy heifers grazing pasture, chicory and plantain in autumn.

Chapter 2

REVIEW OF THE LITERATURE

2.1 Liveweight gain in heifers

2.1.1 Feeding from birth to weaning

Achieving targets for heifer growth and LW in NZ dairy industry is critical for realising high dairy production (Roche *et al*., 2015). These targets must be met to ensure heifers become a profitable part of breeding and milking herd. A critical time during a heifers development is from birth through to weaning as the heifer is developing its internal system to meet the demands after weaning such as growth, reproduction and milk production as well as increasing in body weight. This period of time is important for dairy farmers to concentrate on as there can be high mortality rates and low growth rates if adequate feed is not provided. The nutritional requirements of a heifer must be met immediately after birth and continue for at least 5 weeks (Soberon *et al*., 2012). Most dairy farms in NZ wean heifer calves at around 12 weeks of age.

Soberon *et al*., (2012) found that the greatest positive influence before weaning on a calves lifetime performance was the inclusion of milk into the diet. Most dairy farms in NZ follow a system of once a day feeding of milk with supplementation of hay and concentrates until 12 weeks of age. The heifer's growth and health up to weaning can be affected by the feed type, feed amount and the environment in which it is living in.

The first few hours are vital in a calf's life. They are not born with their own fully functional immune systems and rely heavily on the immunoglobulins passed on from their mother through colostrum to provide passive immunity. At this stage of development it is the type of feed that is critical. Without the colostrum to supply the immunoglobulins milk alone would not support the calf in its development and would result in severe consequences for the heifer. Godden *et al*., (2007), state that the success of a colostrum feeding program will be affected by (1) Quality of colostrum fed, (2) quantity of colostrum fed, (3) quickness of providing the first colostrum feeding and (4) cleanliness of colostrum (i.e. bacterial contamination). Clostral immunoglobulins are absorbed through the calf's intestine. Closure of intestinal permeability occurs spontaneously with age at a progressively increased rate after 12h since birth; mean closure occurred near 24h postpartum (Stott *et al*., 1979). The amount of colostrum fed had no influence on closure (Stott *et al*., 1979). Therefore it is important for a heifer to receive colostrum as early as possible to achieve the greatest intake of immunoglobulins before intestinal permeability decreases. Figure 2.1 display calf immunoglobulin concentrations increasing after feeding until maximum absorption and then decreasing in concentration (Figure 2.1).

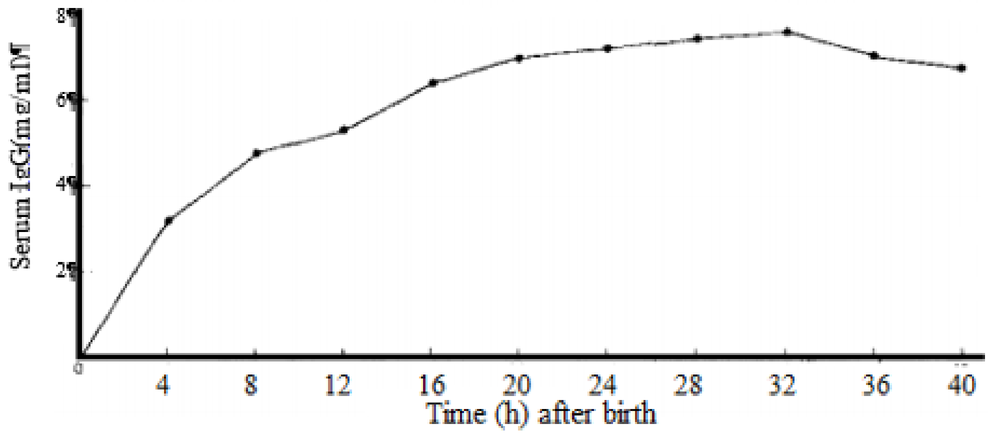

Figure 2.1 Calf immunoglobulin concentrations from birth to 40h (Adapted from Stot. *et al*, 1979)

Colostrum is an important feed for heifers and is necessary for calf health and growth. Table 2.1 displays a summary of Robinson *et al.*'s study of 24h to 48 h serum Ig concentrations on calf survival. The study indicates that of the 43 calves that died in the first 180 d, 19 (44.2%) had concentrations of <12 mg/ml of colostral Ig by 24 to 48 h. Mortality during the first 35 d was very low but markedly increased during each subsequent growth period. Calves with low serum Ig at 24 to 48 h suffered increasing losses with each growth period. Calves with higher serum Ig concentrations (>8 mg/ml) at 24 to 48 h have a greater ability to withstand the harmful effects of the environment (Table 2.1).

Table 2.1 Summary of calf mortality by growth period and categories of serum Ig concentrations at 24 to 48h (Robinson. *et al*., 1988)

Growth period	Ig Group, mg/ml					Total died
	≤12	12.1 to 18	18.1 to 25	25.1 to 40	>40	
0 to 35 d	1	1	1	...	1	4
35 to 70 d	4	4	...	3	...	11
70 to 105 d	6	1	2	2	2	13
105 to 180 d	8	2	1	1	3	15
No. died	19	8	4	6	6	43
No. started	280	157	135	197	231	1000
Mortality, %	6.78	6.09	2.98	3.04	2.59	4.30

Calves fed ad-lib milk will increase in LW faster than those fed a conventional diet (milk fed twice a day at 10%BW) due to the increase in amount of milk consumed, average daily gain for the calves fed ad libitum was 0.8±0.1 kg in the first week after birth, compared to 0.2±0.1 kg for the conventionally fed calves. At weaning there was a significant 10.5 kg weight advantage for the ad libitum-fed calves (Jasper and Weary, 2002). Figure 2.2 displays the increased rate of LW gain from calves fed ad-lib compared to calves fed a conventional diet. Figure 2.2 also shows a lag phase post-weaning (36days). This is due to the calves' rumens being under-developed and not being adapted for the change in diet due to the reduced intake of hay and concentrates associated with ad-lib feeding.

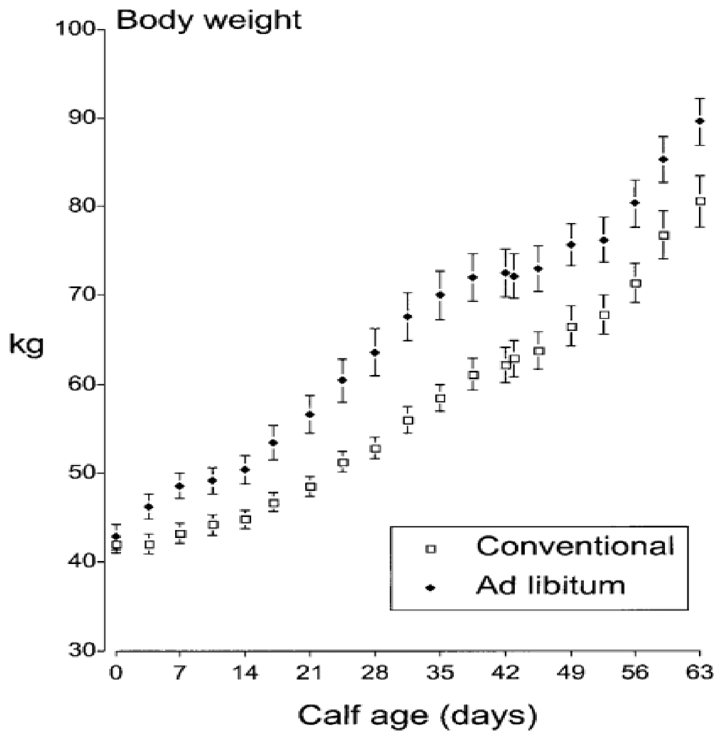

Figure 2.2 The effects of conventional feeding and ad-libitum feeding on liveweight gain of heifer calves (Jasper and Weary, 2002).

The first few weeks are important for LW gain of calves but more so the development of the immune system and digestive structures such as the rumen. The lining of the rumen in a mature animal is covered in villi which greatly increase the surface area to maximise digestion of fibrous feeds such as grass and hay. A calf that is fed only on milk will not develop a rumen layered with villi as milk is easily digested. Adding concentrates and hay to the diet will aid in the development of the rumen therefore decreasing the lag phase once moved to pasture.

In Jasper and Weary's study they concluded that the calves fed ad libitum gained more weight than the conventionally fed calves before weaning, and maintained that weight advantage until at least 9 weeks of age (Jasper and Weary, 2002). They also stated that if the early opportunity for rapid LW gain is not met, high levels of intake later in life may not allow for compensatory growth (Jasper and Weary, 2002).

Issues that can reduce LW gain of calves include scours, pneumonia and naval infection. These disease hazards are greatest while the calf is on liquid feed (Leaver and Yarrow, 1972). With careful management these hazards can be minimised or ideally eliminated and LW gain should not be affected.

In conclusion a heifer calf must receive >12 mg/ml of colostral Ig by 24h to greatly increase its chance of survival. It is necessary for the heifer to receive immunoglobulins via colostrum to develop its immune system. To further the heifer's development LW gain can be positively influenced by high levels of milk from birth. Ad-libitum milk feeding will give a greater rate of growth than conventional feeding, 0.8 kg/day for the first week and 0.2 kg/day for the first week respectively. With the addition of hay and concentrates the heifer will gain weight rapidly whilst also developing a rumen that will adapt well to a change in diet after weaning.

2.1.2 Live weight at first-calving

Studies have concluded that heifers should be 80-90% of their mature weight at calving (Troccon, 1993; NRC, 2001). Most dairy farms in NZ aim for a body condition score of 5.0-5.5 at calving and depending on the age and breed of cow an average production of 356 kg MS/cow during the season with peak milk production between 30-130 days (DairyNZ, 2012). A recent report suggested that up to 70% of the rising two-year old dairy heifers in NZ fail to achieve LW target at mating (McNaughton and Lopdell, 2012), and this was partly attributed to poor quality pasture and low DMI.

The effect of LW at first-calving has shown to have inconsistent results on lactation (Roche *et al.,* 2015). Macdonald *et al*. (2005) reported no effect of a 68 kg difference in first-calving LW in Holstein-Friesian heifers on first lactation milk production, when the difference in LW was established before puberty. However heifers that were 68 kg heavier at first-calving due to differences in post-pubertal growth rates had a 5-7% increase in milk, milk fat and milk protein production during the first lactation (Macdonald *et al.,* 2005). In a NZ analysis, McNaughton and Lopdell (2013) reported a 300 kg increase in first lactation milk yield for a 50 kg increase in first-calving LW. Archbold *et al.* (2012) found the same results in Ireland and furthermore that there was a similar milk production effect in lactation two and three where the yield of milk fat and protein combined were found to increase 1.3 kg during the first three lactations per kilogram of LW at first-calving.

Lower LWs have been shown to delay the onset of puberty. Heifers with a lower weight are less likely to have had an oestrus cycle prior to the planned start of mating. The onset of puberty occurs at the same LW (in general at 43% of mature liveweight) and this demonstrates that age at puberty is related to rate of LW gain (Macdonald *et al.* 2005; Niezen *et al.* 1996; Peri *et al.* 1993).

Heifers at a lower LW take longer to get into calf and therefore potentially becomes a late calver. This is demonstrated in Figure 2.3 showing that for a 30% LW gap potential losses in reproduction in first-calvers of 6% empty rate and a potential 23% of first-calvers in calf later than 6 weeks. A late calving heifer tends to become a late calving cow and this reduces the overall herd performance (Burke *et al.* 2007). Penno (1997) further illustrates the previous point by finding that 20% for heifers grown at below target growth rates were non-cycling at the planned start of mating. In comparison a heifer whose LW is on or above target weights is more likely to have had at least one oestrus cycle if not two by the planned start of mating increasing the chance of an earlier mating. This leads to improved conception rates and improved herd performance. A poor calving pattern results leads to decreased days in milk and problems at subsequent matings (Penno 1997).

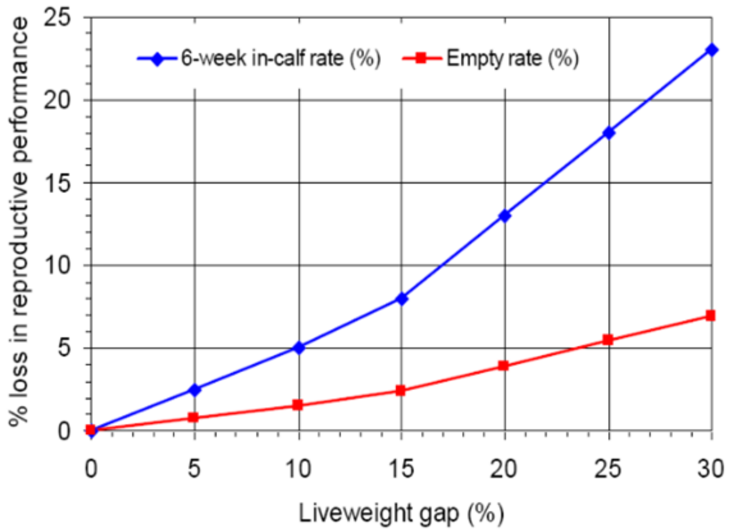

Figure 2.3 Loss in reproductive performance dependent on liveweight (Brazendale and Dirks, 2014)

2.1.3 Mature weight

There is little data available for mature weight of dairy cows in NZ because a limited amount of farmers record mature LW of their cows (Roche *et al*., 2015). Most NZ dairy farmers analyse the condition of their cows from body condition rather than LW (DairyNZ, 2012). The majority of LW data from NZ dairy farms is of 2-year-old heifers for bull progeny tests (Roche *et al.,* 2015). Troccon (1993), recommends that heifers should be at 90% of mature weight at 2-years-old. Depending on the quality and quantity of feed the heifer has received will determine the weight at 2-years-old. Cows that received a poor diet may not reach 90% of mature weight at this stage and therefore 2-year-old LW can only be used as an estimate for mature weight. Archbold *et al*. (2012) reported a linear relationship among 15-month LW, first-calving LW and mature LW; for every 1 kg difference in LW at 15 months, first-calving heifers were 1 kg heavier and mature cows were 1.2 kg heavier

Literature has reported a wide range of mature weights in dairy cows (Troccon, 1993; Otto *et al.,* 1991; Yan *et al*., 1997; Charagu & Peterson, 1998; Coffey *et al.,* 2006). Many factors cause a large range in mature weights. Otto *et al.* (1991), Charagu & Peterson (1998) and Coffey *et al.* (2006) all report mature weights from cows in intensive housed systems (average 620-625 kg). Larger cows produce larger amounts of milk in general, therefore intensive systems tend to have larger cows than less intensive systems (Roche *et al.,* 2015). NZ pastoral systems are far less intensive and dairy cattle grown on these systems have significantly smaller mature weights than those grown in housed systems. NZ Holstein-Friesian cows have an average mature weight of 500-530 kg (Bryant, *et al.,* 2004; Macdonald *et al.,* 2005). Less intensive systems tend to favour smaller cows due to the increase maintenance cost associated with larger cows. NZ, Ireland and Australia all have genetic selection indexes that favour a smaller cow i.e. negative economic weight placed on LW. In order to maintain high production with a smaller cow milk production indexes such as milk protein and milk fat have high economic weights.

Nutritional levels prior to calving are shown to affect the body condition score of the cow, which is an important factor for its short and long term health. Figure 2.4 shows pre calving DMI restriction (4.8 kg DM) significantly lowers body condition score and LW at calving compared to those fed a high DMI (11.9 kg DM) (Roche, 2007). This is due to the extra feed above maintenance being available for the cows own body condition score and not all going to the foetus. Restricted cows lost 0.15 body condition score units during the final 4 weeks precalving, while their LW did not change. In comparison, cows on the high DMI treatment maintained body condition score and gained 32 kg, meaning the low allowance cows calved in lower body condition score than their high allowance comparison (Roche, 2007). Thin cows (body condition score 4 and below) at calving, have an increased risk of mastitis and uterine infections, especially young cows (Dairy NZ, 2012). Also cows fatter than body condition score 6.0 at calving have an increased risk of metabolic disorders around calving (DairyNZ, 2012). Therefore is it critical that targets of body condition score 5.0-5.5 are met to ensure the health of the cows around calving.

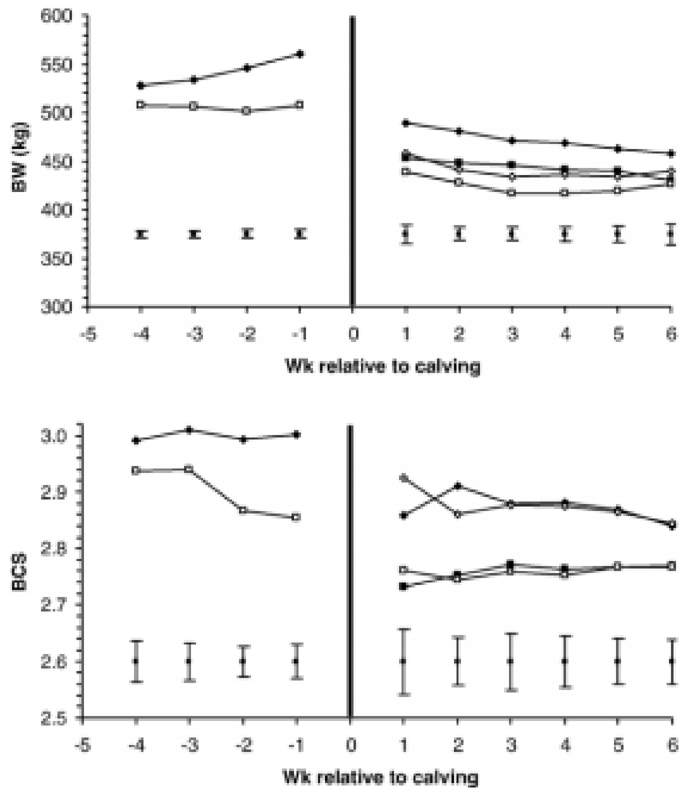

Figure 2.4 Bodyweight (BW) and body condition score (BCS) of cows consuming either 4.8 (± 0.66; Low ▪) or 11.9 (±1.07; High ♦) kg DM for 29 (±7.7) d before calving and either 8.6 (±1.32; Low ▪□) or 13.5 (±2.62; High ♦◇) kg DM for 35 d postcalving. Twice the standard error of the dif*ference is represented by the vertical bar. Similar icons pre- and postcalving represent the mean of cows who remained on the same feed allowance pre- and postcalving (High–High ♦ and Low–Low □). Different icons represent the mean of cows that switched feed allowance treatment at calving (High–Low ◇ and Low–High ▪). (Roche, 2007).*

Key LWs are at weaning, first-calving, and mature weight. The review has shown the importance of these, and also where constraints may be. Heifers may not reach these target LWs due to quality and quantity of feed being low. A solution to this could be the use of alternative forages such as plantain and chicory.

2.2 Effects of chicory and plantain on live weight

Perennial ryegrass requires moist, fertile soil conditions, therefore performing poorly in hot dry conditions when many other deep rooted species continue to perform (Stewart & Charlton, 2003). Perennial ryegrass often contains endophyte which reduces palatability, milk production, LW gain and can have adverse effects on animal health (Bluett *et al.,* 2005). Using alternative forages such as chicory and plantain could be used as a method to minimise urine N concentrations due to lower crude protein content, changes in proportion of rumen degradable versus rumen undegradable protein, increased carbohydrate content, and decreases in fibre content. Previous studies show sheep, beef and deer grazing on swards containing plantain and chicory had higher gain of live weight (LW) than animals grazing on perennial ryegrass-white clover pasture, this has also been found for six-month-old Friesian-Jersey dairy heifers (Handcock *et al*, 2015). Handcock reported that heifers grazing a mixed sward of chicory, plantain and white clover showed to have a LW gain of 0.75 kg/d compared to heifers grazing ryegrass-white clover pasture, which had a LW gain of 0.53 kg/d. The superior growth rates in Handcock *et al.*'s (2015) study on the mixed sward compared to pasture were attributed to a greater ME content (1.1 vs 9.5 MJ/kg DM respectively), CP% (15.2 vs 14.0% DM respectively), organic matter digestibility (78.4 vs 65.4 % respectively) and lower NDF% (25.4 vs 51.0 respectively). DMI is also shown be increased with lower NDF% (Niderkorn & Baumont, 2009), this indicates that DMI was likely higher for the heifers grazing the mixed sward in comparison to the pasture due to a difference in NDF%.

Further studies show that higher LW gains have been assigned to; a lowered internal parasite load (Scales *et al.,* 1995; Barry, 1998; Parish *et al*., 2012), greater DMI (Schreurs *et al*., 2002; Golding *et al.,* 2008; Kenyon *et al*., 2010) and high nutritive values (Burke *et al*., 2002; Kemp *et al*., 2010; Waghorn & Clark 2004). Similarly, lactating ewes (Hutton *et al*., 2011) and dairy cows (Waghorn and Clark, 2004) grazing swards containing chicory and plantain produced more milk than those offered perennial ryegrass-white clover pasture.

Hutton *et al.* (2011) found that ewes grazing a mixed sward of plantain, chicory, red and white clover compared to ewes grazing a ryegrass dominant sward were heavier (P<0.05; 70.9 ± 1.17 kg *versus* 66.1 ± 1.15 kg) and had higher (P<0.05) body condition scores (2.8 ± 0.07 *versus* 2.4 ± 0.07, respectively).

Plantain when fed as a mixed pasture with red and white clover has shown to increase lamb growth rates by 100-150g/d when compared to a ryegrass white clover pasture (Brown *et al*., 2014). The farm that Brown *et al*., (2014) conducted this study on also found that they were able to increase stocking rate on the plantain pasture and had a faster turnover of lambs. A further advantage they found from the plantain pasture was that it results in consistent and predictable animal performance, allowing for improved planning in stock buying and selling management decisions.

Chicory has been shown to have higher nutritive value and higher apparent DM intake compared to perennial ryegrass therefore improving the efficiency of the system through increased milk production (Chapman *et al*., 2008). The results from Chapman *et al.* (2008) were obtained from analysis in south-west Victoria, Australia where conditions are hot and dry. This indicates that chicory performs better than perennial ryegrass under hot and dry temperate conditions (Chapman *et al*., 2008). Similar results were found for plantain on an East Coast dryland farm, NZ (Macfarlane *et al*., 2014). However, a concern with some alternative species to perennial ryegrass is the seasonality of growth (e.g. low growth rates in early spring), and low overall herbage accumulation, when grown as a pure sward (Chapman *et al*., 2014). Moorhead *et al*., (2002) reported lower growth rates of on a pure plantain sward than that recorded by Hutton *et al*., (2011) on a mixed herb sward (222g/d versus 245-298 g/d). A solution for this can be to combine species in a diverse mixture (Sanderson *et al*., 2004). Irrigation can also be used to decrease the effects of seasonality. Kemp *et al*., (2014) reported lower CP concentrations when plantain was water stressed compared to plantain with adequate water (156-175 g/kg DM and 236g/kg DM respectively)

The increased use of irrigation throughout NZ has allowed seasonal effects on pastures to be minimised. Therefore allowing pasture production to increase and with it stocking rates and intensification of dairy farming. This has led to increased N loss from dairy systems and has become a serious concern for many farmers, not just dairy farmers and those under irrigation.

2.3 Nitrogen loss to the environment

N is an essential element for plant growth and necessary for high pasture production on dairy farms. Grazing perennial ryegrass-white clover pasture often provides an excessive amount of N relative to livestock requirements (Pacheco and Waghorn, 2008); in turn, the excretion of surplus N through urination can lead to environmental pollution through greenhouse gas emissions (e.g. nitrous oxide) and nitrate (NO_3^-) leaching to ground water (Di and Cameron, 2002). The leaching of NO_3^- into waterways is considered a serious health hazard, as well as a serious factor in eutrophication (McLaren & Cameron, 1996). The main factors affecting the level of NO_3^- leaching losses are season, climate, land use and soil properties (Di and Cameron, 2002). Waterways with dangerously high levels of NO_3^- (40-100 mg NO_3^--N L^{-1}) that are used as drinking water for stock can interfere with the transport of oxygen in the blood, causing a disorder called methaemoglobinaemia and can cause abortions in cattle (Di & Cameron, 2002).

High concentrations of NO_3^- in drinking water for humans are considered to be particularly harmful to infants younger than 1 year of age. NO_3^- causing methaemoglobinaemia (Cameron *et al.*, 2002). The ingested NO_3^- is converted in the stomach to nitrite (NO_2^-), which is rapidly absorbed into the bloodstream. The NO_2^- causes a reduction in the oxygen-carrying capacity of the blood, and potentially death from cellular anoxia (McLaren & Cameron, 1996). NZ has a standard for the maximum level of NO_3^- allowed in drinking water, 11.3 mg L^{-1} (McLaren & Cameron, 1996).

Urine patches of grazing livestock in particular dairy cows are the main source of leached N from our environment and is a major pollutant of waterways (Betteridge *et al*., 2013; Di & Cameron, 2002; Haynes & Williams, 1993; Ledgard, 2001; Ledgard *et al*., 2009; Pacheco & Waghorn, 2008; Ryden *et al*., 1984). Nitrogen loading from a single urine patch may be up to 1000 kgN/ha (Di & Cameron, 2002). The total amount of N excreted in the urine of a dairy cow is dependent on the amount of times it urinates, the volume of urine excreted and the nitrogen concentration in the urine. Studies have shown a mature dairy cow can urinate between 13 to 73 times/d 5.8 to 54.7L/d and Urinary N concentration ranging between 0.8 to 14.1 mg N/L (Betteridge *et al,* 1986). Betteridge, *et al*., (1986), in a study on grazing steers found that within a 24 hour period, frequency of urination varied from 13 to 73 times, and total daily output ranged between 5.8 and 54.7 L. This is similar to average ranges determined in Seilbie *et al.,* (2015) outlined in Table 2.2. Large variation in urination volume and frequency is evident, both between individual animals and with time of day (Betteridge *et al.,* 1986).

Table 2.2 Characteristics of urine patches deposited by dairy and beef cattle and sheep grazing predominantly pasture-based diets displayed as a range (Adapted from Seilbie *et al.*, 2015).

Study (Location)	*Species (class)*	*Urine N concentration (g N L^{-1})*	*Urination volume (L)*	*Urination frequency (per day)*	*Urine patch size (m^2)*
Haynes & Williams, 1993 (New Zealand)	Cattle (beef/dairy)	8-15	1.6-2.2		0.16-0.49
Aland et al., 2002 (Sweden)	Cattle (dairy)			5-18	
Clarke et al., 2010 (New Zealand)	Cattle (dairy)			11-16	
Lantinga et al., 1987 (The Netherlands)	Cattle (dairy)	6.1-9.7		10-12	
Welten et al., 2013 (New Zealand)	Cattle (dairy)				0.05-0.31
Haynes & Williams, 1993 (New Zealand)	Sheep		0.10-0.18		0.03-0.05
Bristow et al., 1992 (New Zealand)	Sheep	3.0-11.7			

The volume of urine produced is primarily determined by the mineral load that needs to be excreted. Animals fed a high protein diet consume more water and excrete more water in urine (Bannink *et al*., 1999). This is confirmed in other studies, Khelil-Arfa *et al.* (2012), found that the content of crude protein (CP) ingested in daily feed intake was the principal factor affecting the volume of urine. Holten and Urban, (1992) found that urine output was affected by dry matter intake (DM), dietary DM%, and dietary CP (R^2=0.92). In this study concluded that dietary CP was the factor that had the greatest effect on urine volume. In addition to N, urine production is also particularly affected by the excretion of Na and K (Bannink *et al*., 1999).

This review has shown the importance of reducing N loss in the environment and the key contributors leading to N loss. A possible solution to reduce N losses in the environment could be the use of alternative forages such as plantain and chicory.

2.4 Effects of chicory and plantain on nitrogen loss

Plantain and chicory are becoming increasing popular in NZ pastures. They have been shown to have many benefits to animal nutrient intake and performance (Schreurs *et al*. 2002; Chapman *et al*., 2008; Muir *et al*., 2014). Recent studies show pastures containing chicory and plantain may potentially offer a strategy to reduce the environmental impact of livestock farming (Pembleton *et al*., 2015). For example, Deaker *et al*. (1994) and Barry (1998) showed sheep had lower serum urea and rumen ammonia concentrations when fed with plantain and chicory, compared to when they were offered with pasture. Further studies have found that a diverse pasture containing chicory and plantain has been shown to reduce the amount of urea, NH3, creatinine and N% in the urine for lactating dairy cows (Table 2.3).

Table 2.3 Effects of diverse pasture on urine output (Adapted from Totty et al., 2013).

	Ryegrass	*Diverse*	*SEM*	*p-value*
Urea (mmol/L)	180.4	103.5	6.63	<0.001
NH3 (mmol/L)	0.75	0.36	0.06	<0.001
Creatinine (mmol/L)	1.2	0.88	0.05	<0.001
N%	0.58	0.34	0.02	<0.001

Totty *et al.,* (2013) found urea, NH3, creatinine and N in the urine to be significantly lower for cows grazing a diverse pasture containing chicory and plantain. This diverse sward was compared to a standard perennial ryegrass/white clover sward. Offering cows a diverse pasture containing herbs reduced the urinary N concentration by almost half. Milk production for these cows was 2.0 L of milk/cow per day more than cows grazed on ryegrass based pastures. This decrease in N loss and increase in milk production is suggested to be due to a change in N partitioning within the cow.

Previous studies have shown a linear relationship between the excretion of N in urine and N intake (Tas *et al.,* 2006; Hoekstra *et al*., 2007; Higgs *et al*., 2012). The treatments in the study by Totty *et al*, (2013) had no significant difference in N intake however the ryegrass pasture was different in N content to the diverse pasture and had a similar DMI. Totty *et al*., (2013) argued that differences in N intake could be biologically meaningful. The large reduction in urinary N and increased milk production on the diverse pasture indicate the value of reductions in N intake of as little as 50 g of N/d or 8% of N intake. It is suggested that the 2 factors that may have contributed to a change in partitioning are (1) the ratio of WSC and CP and (2) the possibility of bioactivity of the secondary plant compounds known to exist in herbs.

In similar studies conducted indoors (Woodward *et al*., 2012), milk yield and N partitioning to milk, urine and faeces, were compared in dairy cows fed either a standard perennial ryegrass white clover pasture or a diverse pasture which also contained chicory, plantain and lucerne. Cows fed on the diverse pasture had higher milk yield and milk N output when compared with the standard pasture (12.5 vs 11.3 kg/cow/day; 79 vs 68 g milk N/cow/day respectively), and had half the total urinary N output than cows fed the standard pasture (100 vs. 200g N/cow/day). It is suggested that the difference is explained by the difference in total feed N intake (350 vs 466 g N/cow/day), and lower faecal N output (136 vs 151 g N/cow/day for diverse and standard pasture respectively) of cows fed diverse pasture (Chapman *et al*., 2014). A limited number of studies have investigated which herb species and how much of each species is needed to achieve a reduction in UN from livestock, without losing productivity.

The conclusions from these literature reviews are:

- Key LW's for weaning, first-calving and mature weight of cows are often not met in pastoral New Zealand dairy systems due to insufficient levels and quality of feed.
- Chicory and plantain have been shown to have increase LW gain of heifers when in comparison to traditional ryegrass-white clover pastures.
- Nitrogen loss primarily through urine patches, is an increasing concern for dairy farms in New Zealand due to negative impacts on the environment.

- Chicory and plantain have been shown to reduce N losses to the environment when compared to traditional ryegrass-white clover pastures.

The objective of this study is to determine the effect of the proportion of plantain and chicory in the diet with pasture on LW gain, N losses in urine and grazing behaviour of dairy heifers in autumn.

Chapter 3

MATERIALS AND METHODS

3.1 Ethical Statement

All procedures were approved by Lincoln University Animal Ethics Committee (**AEC** 557), New Zealand.

3.2 Experimental Design and Animal Management

The study was conducted at Ashley Dene Pastoral Systems Research Farm, Lincoln, New Zealand (latitude: -43.65; longitude: 172.32). Pure swards of chicory (cultivar Choice) and plantain (cultivar Tonic), and a binary pasture mixture of perennial ryegrass (cultivar Prospect) and white clover (cultivar Weka) were sown as adjacent strips on 15 January 2014. The study was conducted over a period of 32-d, from 7 April to 8 May 2015. Plots were grazed to low herbage mass (1200 kg DM/ha) 40 days prior to start of each trial and then fertilised with 50 kg N/ha as urea. Heifers were adapted to their treatments over the first 7 d (acclimation period), by gradually increasing the herb allowance to desired level, so that total herbage allowance was achieved. After the acclimation period, the study had a 25-d experimental period.

Sixty Friesian x Jersey heifers aged 8-9 months were blocked based on Breeding worth, breed and body weight. All heifers grazed on a perennial ryegrass-white clover pasture as one herd prior to the study. Heifers (LW = 176 ± 13.9 kg, breeding worth = NZ $171 ± 29.1; mean ± SD) were allocated into five dietary treatment groups: 100% perennial ryegrass-white clover pasture (R; n = 12); 50% pasture + 50% chicory based on area of feed allocated (50%C; n = 12); 50% pasture + 50% plantain based on area of feed allocated (50%P; n = 12); 75% pasture + 25% chicory based on area of feed allocated (25%C; n = 12); and 75% pasture + 25% plantain based on area of feed allocated (25%P; n = 12). All heifers received one dose of anthelmintic (Genesis Pour-on, abamectin 10g/L, Ancare, Australia) treatment in March 2015.

Heifers were offered a fresh herbage break every 3 days at 0900 h. For herbage allowance calculation, post-grazing herbage mass was assumed to be 1500 kg DM/ha for pasture and 1000 kg DM/ha for chicory and plantain. Herbage allowance was calculated to meet the metabolisable energy (ME) requirements for maintenance plus a 1.0 kg daily LW gain (Nicol and Brookes, 2007): Herbage allowance (kg DM/d) = [0.65 x LW (kg)$^{0.75}$ + 30 (MJ ME)] / 11.5

(MJ ME/kg DM). Each group was provided a portable water trough to allow *ad libitum* access to water.

3.3 Herbage measurements

3.3.1 Herbage Mass and Apparent Intake

Compressed pasture height was measured pre- and post-grazing for pasture, chicory, and plantain, using a rising plate meter (RPM; Jenquip, Fielding, New Zealand). The relationship between compressed height and herbage mass was estimated prior to commencement of the study, by cutting 40 (n = 20 pre-grazing and 20 post-grazing quadrats) quadrats, each 0.2 m^2 to the ground-level, in pasture, chicory, and plantain. Immediately prior to cutting, one RPM height measurement was recorded from the area to be harvested. Herbage samples were oven-dried at 65°C for 48 h and weighed. Linear regression equations between compressed height and herbage mass were then developed for each species, by fitting a single line. The calibration equations between herbage mass and RPM (0.5 cm unit) used were:

Perennial ryegrass-white clover pasture (kg DM/ha) = 129.5 × RPM – 67.2; R^2 = 0.80; $P< 0.001$

Chicory (kg DM/ha) = 77.9 × RPM + 699.9; R^2 = 0.74; $P< 0.001$

Plantain (kg DM/ha) = 69.0 × RPM + 873.6; R^2 = 0.71; $P< 0.001$

A total of 30 RPM measurements from each species in each treatment were made immediately before and after each grazing. The mean apparent DMI was calculated for each 3 d allocation based on the product of the difference between pre- and post-grazing herbage mass, and area grazed, divided by the number of heifers. The apparent DMI was corrected for regrowth of herbage over 3 d. The regrowth of herbage was quantified for each species by taking 20 RPM measurements from a 2 m^2 area of each forage species on d 4, 11, and 20 after grazing, and using this to calculate average re-growth rate.

3.3.2 Herbage Botanical Composition and Nutrient Content Analysis

A total of 15 herbage samples (0.1 m^2) were cut to post-grazing level from each species in each treatment before forage allocation. The samples were used to estimate the DM%, botanical, and chemical composition of different treatments. Each herbage sample was then divided into 3 sub-samples. The first sub-sample was weighed fresh, dried in an oven at 65°C for 48 h, and re-weighed to determine the DM%. The second sub-sample was separated into sown species, weeds, and dead material before oven-drying at 65°C for 48 h, and weighing. The percentage botanical composition on a DM basis was then determined. The third sub-sample was freeze-dried and ground to 1 mm for analysis of nutrient content. Near infrared reflectance spectrophotometry (Foss NIRSystems 5000, FOSS NIRSystems Inc, USA) was used to estimate N, OM in DM (DOMD), and NDF (Corson *et al*., 1999). The ME was calculated from predicted DOMD [ME (MJ/kg DM) = DOMD (g/kg DM) × 0.016], on the basis of an *in vitro* cellulase digestibility assay (Roughan and Hollan, 1977; Dowman and Collins, 1982), which had been calibrated against *in vivo* standards (Corson *et al.,* 1999). To calculate the nutrient intake of the nutrient content of the pre-grazing herbage of treatment groups containing both herb and pasture the following calculating was used:

Nutrient intake = (herb DMI × herb nutrient content + pasture DMI × pasture nutrient content) ÷ (total DMI)

3.4 Animal measurements

3.4.1 Live Weight Gain Measurements

All heifers were weighed after a 12 h fast on d 1, d 16 and d 25 of the experimental period. The LW gain was calculated as:

LW gain (kg/d) = LW change (kg) ÷ number of days of study (d)

3.4.2 Urine and Plasma Measurements

On experimental d 11 and d 23, heifers were herded into the cattle yards at 1200 h. One urine sample was collected for each heifer mid-stream following vulva stimulation, and acidified immediately below a pH of 3.0 with concentrated sulphuric acid to minimise ammonia volatilization. One 10 mL blood sample per heifer was collected from the coccygeal vein using sodium heparin vacuette tube (Greiner Bio-one, Kremsmunster, Austria). The blood was then centrifuged at 3,000 × *g* at 4°C for 15 min to harvest plasma. Urine and plasma samples were then stored at −20°C until analysis.

Urine and plasma analyses were performed by Lincoln University Analytical Services (Lincoln University, Christchurch, New Zealand). Concentrations of allantoin in urine was analysed following the method using HPLC (Agilent 1100 series, Waldbronn, Germany) as described by George *et al*., (2006). Urinary N concentration was determined by N analyser (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany). Urine urea and creatinine, plasma urea N

(PUN) and glucose were analysed using a Daytona RX Clinical Analyser (Randox, Nishinomiya, Japan).

3.4.3 Estimation of Urinary Nitrogen Excretion and Rumen Function

The Urinary N was estimated from PUN and LW measurements using the equation published by Kohn *et al*., (2005):

Urinary N (g/d) = 1.3 × PUN (g/L) × [LW at the start (kg) + LW at the end (kg)] ÷ 2

The microbial protein synthesis index (MPS) was estimated using urinary concentrations of allantoin and creatinine according to Gonda *et al*., (1995):

MPS (mmol/mmol) = Urinary allantoin (mmol/L) ÷ Urinary creatinine (mmol/L)

3.4.4 Grazing Behaviour Measurements

On experimental d 4 and 20, the heifers were recorded as grazing, ruminating or idling at 15 minute intervals over the first 2 h following fresh herbage allocation and then for a further 2 h at 1500 h, bite rate per minute was also recorded during these times. On experimental d 5 and 21, grazing behaviour and bite rate were recorded over 2 h commencing at 0700 h. Grazing behaviour was visually recorded for each heifer by trained observers. The average from all observations was used for analysis.

3.4.5 Urination Behaviour Measurements

On experimental d 4 and 20, urination frequency was recorded over the first 2 h following fresh herbage allocation and then for a further 2 h at 1500 h. On experimental d 5 and 21, urination frequency was recorded over 2 h commencing at 0700 h. Urinations were visually recorded for each heifer by trained observers. The average urination frequency from all observations was used for analysis.

3.4.6 Water intake measurments

A water meter (Manufacture part number - GG-L12ASC02B051 from Altecnic Ltd) was installed into the trough in each treatment to obtain the water intake from the trough. Readings were taken d 3, 10 and 18. Water intake from forage for each treatment was calculated using the following equation:

Water from forage (l/d) = (DMI ÷ DM%) - DMI

Total water intake was then calculated by adding trough intake to forage intake.

3.4.7 Urine Patch Size Measurements

On experimental d-22 urine patch size was recorded in each treatment between 0900h and 1100h. Immediately after an urination event was finished a line of paint was sprayed around the perimeter of the urine patch. A meter ruler was placed next to the sample to use as a calibration factor. A digital photograph was then taken from above the sample and ruler. Care was taken to ensure all patch edges and the entire ruler were included in the photograph. Ten photographs were recorded for each treatment. The area of each patch was then calculated using SketchAndCalc™ Version 4.1.3. The scale of the photograph was set using the ruler in the photograph, as the known length of this was 1.01m. The equation used to determine urine patch N load was:

$$\text{Urine patch N load (kg ha}^{-1}\text{)} = ((\text{Urine N concentration (g N L}^{-1}\text{)} \times \text{Average urination volume (L)}) \div \text{Calculated average urine patch area (m}^{2}\text{)}) \times 10$$

3.4.8 Urine Volume Calibration Measurements

Urine volume per urine patch was calculated from the urine patch size. A known volume of water was poured - at the height of a heifer's vulva - onto the soil. The wetted area then outlined and photographed with a ruler and measured using the same protocol as described for Urine Patch Size Measurement. Five volumes were used: 250, 500, 1000, 1500 and 2000ml. Each volume was replicated five times for pasture, plantain and chicory.

3.5 Data Analysis

Urine, plasma, LW gain, behaviour and urination data were averaged across sample days before analysis. Urine, plasma, LW gain, behaviour and urination data were then analysed by one way ANOVA using Genstat (version 15.1; Payne *et al*., 2015), including dietary treatment as a fixed effect. A multiple comparison test (Student-Newman-Keuls Test) was performed to differentiate the mean values among treatments effect of ANOVA was significant ($P < 0.05$). Excel was used to calculate a regression equation for the urine volume calibration measurements for pasture, plantain and chicory. An equation was chosen with the strongest R^2 value for each forage.

Chapter 4

RESULTS

4.1 Herbage data

Table 4.1 shows herb and pasture mass and nutritive average values for each treatment from the 32 day trial period. Pre grazing mass ranged from 2029 kg DM/ha to 2671 kg DM/ha and post grazing mass ranged from 883 /ha to 1529 kg DM/ha. ME and DOMD was high in all treatments (>12.1 MJME/kg DM & 75.8 % respectively). CP averaged 18.6% for pasture area and tended to be lower in chicory (13.5%) and plantain (13.0%). ADF averaged 25.2% for pasture area and tended to be lower in chicory (20.1%) and plantain (21.2%). NDF averaged 44.6% for pasture area and tended to be lower in chicory (22.4%) and plantain (29.2%). DM averaged 19% for pasture area and tended to be lower in chicory (13%) and plantain (14%).

Table 4.1 Herb and pasture mass and nutritive values for all treatments, mean ± SEM

	R	*50% C*		*25%C*		*50%P*		*25%P*	
		R	C	R	C	R	P	R	P
Pre grazing mass (kg DM/ha)	2596±101.3	2029±166.8	2352±187.0	2398±125.2	2465±239.7	2671±208.2	2448±177.3	2423±146.4	2431±161.1
Post grazing mass (kg DM/ha)	1242±106.6	883±66.4	1283±64.5	1232±66.8	1335±55.9	1108±52.0	1344±32.8	1305±74.5	1529±105.4
ME (MJME/kg DM)	12.3±0.19	12.2±0.08	13.0±0.15	12.2±0.10	13.0±0.10	12.3±0.10	12.8±0.2	12.1±0.1	12.7±0.21
DOMD (%)	76.6±1.21	76.3±0.47	81.0±0.91	76.13±0.64	81.0±0.60	76.9±0.64	79.9±1.02	75.8±0.82	79.2±1.28
CP (%)	19.4±1.21	18.9±0.48	13.4±0.71	17.8±0.64	13.5±0.76	19.0±1.21	13.3±0.58	17.7±1.08	12.7±0.63
ADF (%)	25.1±0.98	24.9±0.46	20.0±0.46	25.3±0.58	20.1±0.36	24.7±0.33	21.5±0.90	25.8±0.66	20.8±0.70
NDF (%)	45.6±1.72	43.5±1.19	22.7±0.67	44.6±1.15	22.1±0.71	44.0±0.80	29.7±3.13	45.4±1.20	28.7±1.99
DM (%)	18±0.5	18±0.6	13±0.3	19±0.5	13±0.5	19±0.7	14±0.03	19±0.8	14±0.3

ME = Metabolisable energy, DOMD = Digestible organic matter in the dry matter, CP = crude protein, ADF = Acid detergent fibre, NDF = Neutral detergent fibre, DM = dry matter.

4.2 Animal data

4.2.1 Liveweight and blood and urine compostion

Table 4.2 shows the average LW gain of heifers in each treatment from start to end. The chemical composition of urine and blood samples taken on days 11 and 23 of the trial is also displayed in Table 4.2. LW gain averaged 0.70 kg/d across all treatments. LW was lower (P = 0.11) in 50%C than the other treatments, which all had similar LW gain. There was no significant difference in urine N, NH3, BUN, glucose, urea, faecal DM and microbial protein synthesis between all treatments (P>0.05).

Allantoin was significantly lower in R and 25%P (2.45mmol/L and 2.43mmol/L respectively) and higher in 25%C (4.17mmol/L) (P=0.029). Uric acid was significantly lower in R and 25%P (0.17mmol/L and 0.16mmolL respectively) and higher in 25%C (0.28mmol/L) (P =0.033). A small difference was apparent in creatinine, where R and 25%P were lower (0.75mmol/L and 0.78mmo/L) and 25%C was higher (1.18mmol/L) (P=0.07).

Table 4.2 Average LW gain and chemical composition of urine and blood samples for heifers in each treatment

	R	*50%C*	*25%C*	*50%P*	*25%P*	*LSD*	*P-value*
LW gain (kg/day)	0.77	0.47	0.83	0.74	0.69	0.282	0.110
Urine N (%)	0.15	0.17	0.23	0.17	0.18	0.068	0.230
NH3 (mmol/L)	0.66	1.04	0.99	1.68	2.24	1.561	0.278
Allantoin (mmol/L)	2.45b	3.53ab	4.17a	3.47ab	2.43b	1.251	0.029
BUN (mmol/L)	8.15	7.70	9.03	7.25	8.91	1.561	0.117
Glucose (mmol/L)	5.32	4.80	4.76	4.70	4.71	0.585	0.185
Uric acid (mmol/L)	0.17b	0.23ab	0.28a	0.20ab	0.16b	0.081	0.033
Creatinine (mmol/L)	0.75	0.95	1.18	1.08	0.78	0.348	0.070
Urea (mmol/L)	32.61	34.60	47.53	35.89	39.50	17.337	0.457
Faecal DM %	0.116	0.124	0.122	0.124	0.100	0.0218	0.151
MPS (mmol/mmol)	3.30	3.71	3.43	3.28	3.25	0.512	0.355

BUN = Blood urine nitrogen, MPS = Microbial protein synthesis

4.2.2 Behaviour and urination

Figure 4.1 shows the calibration curves used to determine urine volume from urine patch size for each forage. A power relationship was used to create the strongest R^2 values. Each curve showed a strong positive relationship (>0.85)

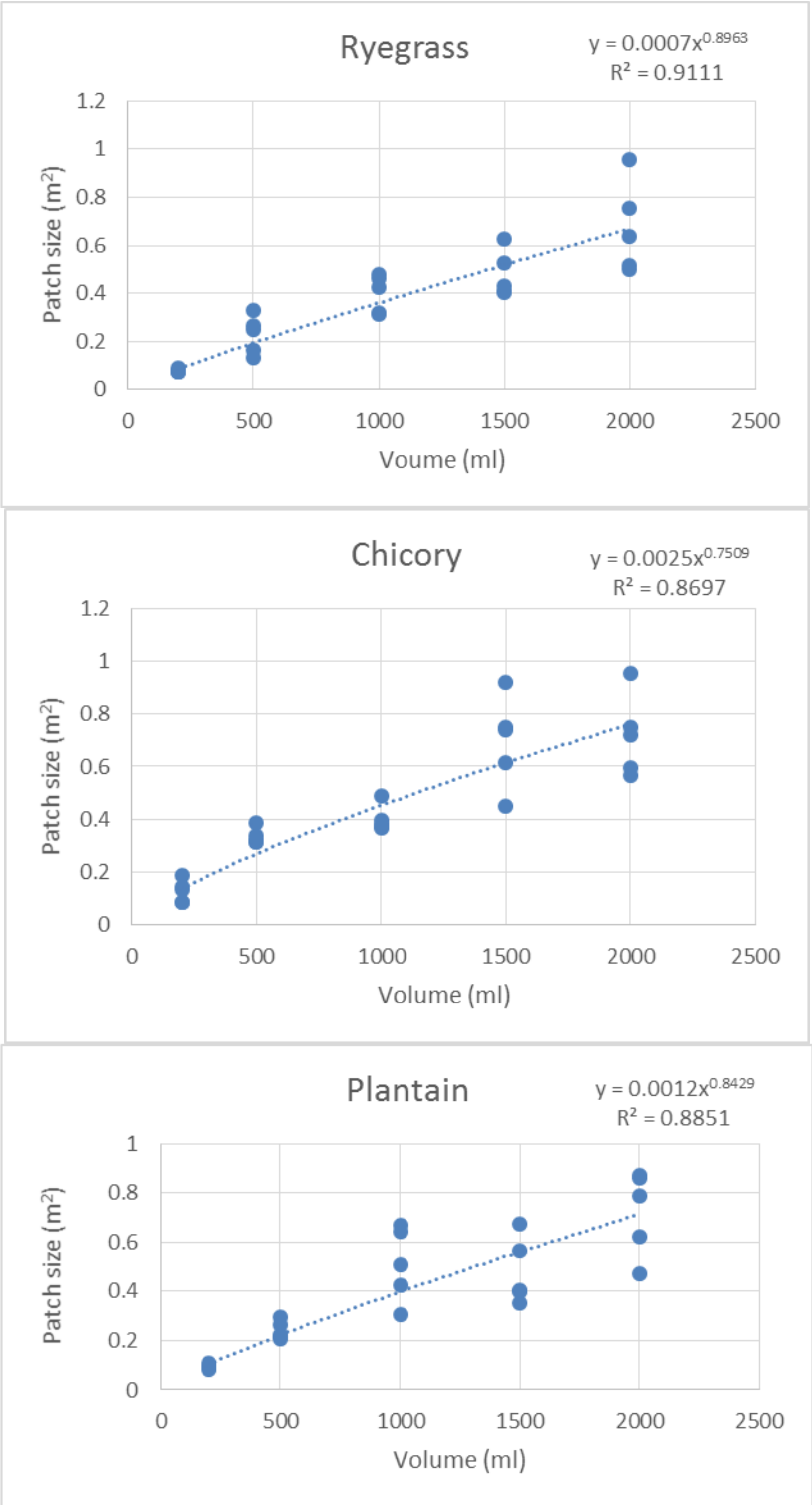

Figure 4.1 Calibration curves for the relationship between urine patch size (m2) and volume (ml) for three pasture types

Table 4.3 shows the grazing and urination behaviours of heifers in each treatment averaged from six 2 hour periods, with urine patch size measurements taken on day 22. There was a significant difference in time spent grazing between treatments ($P<0.001$). 50%C and 25%C spent the longest time grazing, 242.5 minutes/hf/6hrs and 240.6 minutes/hf/6hrs respectively. 50%P spent the least time grazing 200.6 minutes/hf/6hrs and the other treatments were in between these treatments.

50%C and 50%P spent the least time ruminating, 16.9 and 13.1 minutes/hf/6hrs respectively. 25%P spent the greatest time ruminating, 48.1 minutes/hf/6rs, which was significantly greater than all other treatments ($P<0.001$). 50%P had a significantly greater length of time spent idling, 146.2minutes/hf/6rs than the other 4 treatments ($P<0.001$). The average time spent idling for other treatments was 103.0 minutes/hf/6hrs. Bite rate ranged from 31 bites/minute/hf (50%C treatment) to 44 bites/minute/hf (R treatment). There was a significant difference between treatments ($P<0.001$). There was a significant difference in the number of urinations between treatment ($P<0.001$). R has the greatest amount of urinations 5.91 urinations/hf/6h. 50%C has the least amount of urinations 2.33 urinations/hf/6h. The other treatments ranged from 3.58 urinations/hf/6h to 4.17 urinations/hf/6h.

Table 4.3 Grazing and urination behaviour of heifers in each treatment

	R	*50%C*	*25%C*	*50%P*	*25%P*	*LSD*	*P-value*
Grazing min/hf/6h***	231.1ab	242.5a	240.6a	200.6c	208.8bc	22.76	<0.001
Ruminating min/hf/6h	35.0b	16.9c	23.1bc	13.1c	48.1a	11.13	<0.001
Idling min/hf/6h	111.9b	100.6b	96.2b	146.2a	103.1b	24.16	<0.001
Bite rate bites/minute/hf	44a	31d	39ab	37bc	33cd	5.7	<0.001
Urination frequency Urinations/hf/6h	5.91a	2.33c	3.58b	4.00b	4.17b	1.06	<0.001
Urine patch area /urination (m^2)	0.309	0.325	0.217	0.271	0.228	0.0890	0.071
*Urine volume/ urination (ml) ****	839	834	560	719	596	253.5	0.092

*heifer; **hour; ***Calculated from area based on equation in Fig 4.1

Table 4.4 shows the apparent DMI, N intake and water intake of heifers in each treatment. There was no significant difference in the DMI or apparent N intake between treatments ($\tilde{x}$=4.76 kg/d; P=0.836 and $\tilde{x}$=0.12 kg/d; P=0.233, respectively).

The apparent water intake from forage showed no significant difference between treatments ($\tilde{x}$=23.94l/d; P=0.998). There was a significant difference in the water intake from the trough (P=0.024). 50%C consumed less water from the trough than the other treatments (1.03l/d). There was no apparent difference in the total water intake between treatments ($\tilde{x}$=26.99).

Table 4.4 Average DMI, N intake and water intake of heifers in each treatment

		R	*50%C*	*25%C*	*50%P*	*25%P*	*LSD*	*P-value*
DMI (kg/d)		4.99	4.41	4.78	4.64	4.96	1.15	0.836
Apparent N intake (kg/d)		0.15	0.11	0.13	0.11	0.12	0.04	0.233
Apparent water intake	*From forage (l/d)*	24.60	24.00	23.73	23.63	23.76	6.26	0.998
	From trough (l/d)	3.36b	1.03a	3.64b	3.06b	4.13b	1.77	0.024
	Total (l/d)	27.96	25.03	27.37	26.69	27.89		

Chapter 5

DISCUSSION

5.1 Live Weight gain

LW gain averaged 0.70 kg/d for all treatments. This is greater than what has been reported by Clifford *et al.,* (2014) on ryegrass and less than the results found by Handcock *et al.,* (2015) on chicory, plantain and white clover. Both of these studies used six-month-old dairy heifers. However this study by Clifford *et al*., (2014) reports a LW gain from ryegrass (0.57 kg/d) which would understandably be lower than the current study due to lower nutritive values and DMI. Handcock *et al*., (2015) an average LW gain of 0.75 kg/d, this marginally greater LW gain could be due to a higher CP% of chicory, plantain and clover when compared to the chicory and plantain in the current study (15.2 vs 13.13.3% respectively)

The NZ industry target for heifers at the same age is 0.53 kg/d (Handcock *et al*., 2015). Increased ME has been shown to increase LW gain in six-month-old dairy heifers. In Handcock *et al*.'s (2015) study comparing a mixed sward of chicory, plantain and white clover to a ryegrass-white clover pasture higher LW gains were attributed to a greater ME content (1.1 vs 9.5 MJ/kg DM respectively), organic matter digestibility (78.4 vs 65.4 % respectively) and lower NDF% (25.4 vs 51.0 respectively). DMI is also shown be increased with lower NDF% (Niderkorn & Baumont, 2009), this indicates that DMI was likely higher for the heifers grazing. There was no difference in the ME, DMI and digestibility for all treatments in the current study therefore it is understandable to find no difference in LW gain between treatments.

Estimated ME intake was 61.4, 55.6, 59.3, 58.2 & 57.6 MJME/hf for R, 50%C, 25%C, 50%P and 25%P respectively. Estimated CP intake was 968, 712, 799, 749 and 816 g/hf for R, 50%C, 25%C, 50%P and 25%P respectively. This corresponds to a CP% of 19.4, 16.2, 16.7, 16.2 and 16.5% in the diet for R, 50%C, 25%C, 50%P and 25%P respectively. CP intake from all treatments was sufficient to support the target LW gain of growing heifers in this study, estimated to be 15-16% of DMI (Pacheco and Waghorn, 2008). This result is higher than that of Handcock *et al*., (2015) where average CP was 14.6%. This would suggest that a diet of chicory or plantain alone would not have sufficient CP levels to sustain LW gain in heifers as average CP for chicory and plantain in this trial was 13.5 and 13.0% respectively.

There was no negative effect of feeding chicory or plantain at 25 and 50% of the grazing area offered on growth of heifers in this study in autumn compared to a ryegrass/white clover pasture. Cheng *et al*.'s (unpublished) results showed a lower LW gain for dairy heifers in both spring and autumn where the diet was pure chicory (CH) compared to chicory plus pasture (PA+CH) or pasture only (PA). This was particularly pronounced in autumn where heifers grazing CH gained only 0.03 kg/day over the 28-d trial period. Previous studies indicate the effect of feeding chicory on livestock performance to be variable. Higher LWG in sheep (Kenyon *et al.,* 2010), heifers (Handcock *et al.,* 2015), bull calves (Berry, 2013) and deer (Schreurs *et al.,* 2002), and milk production (Waugh *et al*., 1998; Chapman *et al*., 2008) in lactating dairy cows, has been demonstrated for animals grazing chicory than perennial ryegrass-white clover pasture. However, lower LWG has also been recorded for deer (Hoskin *et al*., 2003) and heifers grazing 100% chicory compared to ryegrass (Cheng *et al,* unpublished). Further, in a recent study including chicory in the perennial ryegrass diet as a mixture of pure sward did not improve dairy cow milk production over perennial ryegrass pasture (Muir *et al*., 2014). The reason for a lack of effect of chicory and plantain on LW gain is most likely due to the difference in DMI being none significant. In contrast to past studies (Handcock) ME was similar, so that total ME intake was similar between treatments. This contrasts dryland studies where ME was greater in chicory and plantain than perennial ryegrass due to reduced performance of ryegrass under water stressed conditions.

Positive effects of chicory and plantain on LW gain have been reported for lambs when compared to perennial ryegrass. Fraser & Rowarth, (1996) reported LW gains ranging from 181 to 214 g/d on chicory and 8 to 141 g/d on plantain compared to 98 to 136 g/d on ryegrass. Low levels of tannin have been detected in chicory (0.42% of the DM) and it has been suggested that the high growth rates of ruminants fed chicory may be due to tannin protecting protein from degradation in the rumen (Terrill *et al.,* 1992). Plantain is also known to contain tannin (Diirfler & Roselt, 1989, Launert, 1984) and further to have antibiotic properties (Grieve, 1931). It is reported to be highly palatable to sheep and cattle (Milton, 1933 & Ivins, 1952), and have a high mineral content (Thomas *et al*., 1952) therefore supporting high growth rates (Deaker *et al*., 1994).

5.2 Grazing behaviour

A significant difference was observed in the minutes spent grazing, ruminating and idling between all treatments ($P<0.001$). Bryant *et al*., (2012) found that mixed aged dairy cows grazing simple swards of ryegrass spent longer grazing than cows grazing diverse swards containing chicory and plantain. Grazing behavioural correlations involve trade-offs, for example in time allocation (Gregorini *et al*., 2015). Cows cannot ruminate or idle while they are grazing, and vice versa. Although all these activities are important, it is impossible for an animal to maximize each. Therefore, an adaptive trade-off on demands for grazing, ruminating, and idling is necessary (Gregorini et al., 2015). The largest proportion of the daily herbage DMI in grazing dairy cows happens during the first few hours (3–4 h) after new pasture is allocated (Gregorini *et al.,* 2009). This study recorded behaviour over 3 different time periods during the day to account for variances in grazing time.

Grazing behaviour was significantly affected by pasture type. Minutes spent grazing was the same in chicory treatments, which were significantly great than the plantain treatments (mean = 241 vs 204 minutes/6h); there was no significant difference between plantain treatments. R treatment had a grazing time of 231 minutes/6h, this was not significantly different from the chicory or plantain treatments. Heifers grazing pasture had a greater bite rate than heifers grazing pasture containing chicory and plantain (44 vs 35 bites/minute respectively). This result is similar to previous studies by Bryant *et al.,* (2012) who found bite rate of mixed aged cows were faster on grass-based pasture (49 bites/minute) than pasture containing herbs (44 bites/minute). This difference in bite rate is most likely due to increased stem in the treatments containing herb. Increased stem could have constrained bite rate by increasing handling time, or variation in choice of species could have slowed bite rate due to grazing selection (Rutter 2006). Heifers will adjust grazing strategy to optimise intake (Bryant *et al*., 2012). Swards containing herbs have different sward architecture to pasture and this may affect a cow's foraging strategy as sward height is depleted. A decreased sward height is shown to decrease the bite depth and bite mass (Bryant *et al.*, 2012). Previous studies show bite mass to be the main driver for intake rate due to cows altering their grazing behaviour to increase intake per bite (Hodgson 1985; Newman *et al*. 1994). As grazing time is important in determining total intake when intake rates are similar (Dillon, 2006) the similarity in total apparent DMI between treatments would indicate that the heifers may have altered their grazing time to maintain intake rate as there was no evidence of variation in bite mass between treatments.

5.3 Nitrogen loss

There was little difference in the N concentration of urine, or of BUN. Differences in urine N concentration might occur due to differences in N intake or water intake, or mineral load. Studies have found that the volume and N concentration of urine produced is primarily determined by the mineral load that needs to be excreted (Bannink, *et al*., 1999 & Khelil-Arfa, *et al.,* 2012). However, N intake and total water intake did not differ between treatments, therefore the effect expected is to be negligible. It is surprising given this that urine frequency was greater in R than other treatments. The exact mechanisms are unclear, but may reflect differences in mineral intake, or in water intake temporal patterns throughout the day. Animals fed a high protein diet consume more water and excrete more water in urine (Bannink *et al*., 1999). This theory is confirmed in other studies, Khelil-Arfa *et al.* (2012), found that the content of crude protein (CP) ingested in daily feed intake was the principal factor affecting the volume of urine. In addition to N, urine production is also particularly affected by the excretion of Na and K (Bannink *et al.*, 1999). Cheng et al., (unpublished) found the concentration of Ca and Na to be higher in chicory than pasture. High intake of Ca and Na has been linked to frequent urination events (Deaker *et al.,* 1994). Other studies have noted a role of secondary plant compounds in determining urination frequency (Deaker *et al.,* 1994; Tamura and Nishibe, 2002), and whether subtle changes in these created the differences observed among treatments and between seasons needs to be researched further

Urine patch size was on average 0.27 m^2 this is smaller than reported in other studies (Table 2.2). Moir *et al*., (2010), also reported an average urine patch size form mature dairy cows of 0.34 m^2 across three years, Richards and Wolton, (1976) reported 0.49 m^2 as an average from mature dairy cows and eighteen-month-old Friesian steers. This difference is due to the difference in live weight of animal used which would therefore influence the urine volume, concentration and urine patch area. Also in the current study urine patch size was only calculated from 0900-1100h and therefore may give a different result than average patch size over 24h.

Urine N concentration ranged from 1.1g N L^{-1} to 1.4g N L^{-1}. This range is lower than previous estimates. Betteridge *et al.* (2013) found that the average load of N on pasture was 8.6g N L^{-1}, and Pacheco *et al.* (2010) found an average concentration of 5.7g N L^{-1} on pasture, varying depending on season and species. Both of these studies were measuring urine N concentration from mature cattle. It is also worth noting that Betteridge *et al.* (2013) found that not all urination events had the same concentration, the most concentrated urine was excreted during the night. The most commonly cited reference for urine N loading rate is Haynes and Williams (1993), who assumed an average N load of 1000 kg N ha^{-1} for a mature aged dairy cow, which consisted of: urine N concentration of 10 g N L^{-1} , urination volume of 2 L, and urine patch surface area (wetted) of 0.2 m^2. Based on urine volumes found, patch area and N concentration measured, the N loading on a single urine patch would range from 40.7 kg N ha^{-1} to 59.4 kg N ha^{-1}. This is in the range between figures reported by Selbie *et al.,* (2015) for beef cattle and sheep (Table 2.2). This is understandable considering the size and age of animals used in the current trial in comparison to a mature beef animal and a sheep. Further the urine N concentration was much lower than previous studies as was the area and volume (Table 2.2). The N loading in each urine patch is assumed to be the same between treatments in the current trial due to no difference in N concentration or volume. However due to the significant difference in urination frequency it indicates that heifers grazed on perennial ryegrass excrete more total N than heifers on chicory or plantain. Heifers on R treatment urinated on average 5.9 urinations/hf/6h and the heifers on the other treatments urinated 3.5 urinations/hf/6h, both at an average calibrated volume of 601ml urination. The mean urination frequency and mean volume was less than that reported by Selbie *et al.* (2015) (Table 2.2) who reported studies that used mature dairy cows. The difference would be due to the difference in liveweight of the animals. As animal liveweight increases the volume of urine excreted is shown to also increase (Selbie *et al.,* 2015).

Urination frequency was only measured over six hours. If differences in urination frequency remain for remaining 18hours of the day then total N loading from urine patches over 24 hours, assuming constant area allocation for 24 hours would be 963 kg N ha^{-1} for R and 691 kg N ha^{-1} for the other treatments. This is lower than Haynes and Williams (1993) study which stated 1000 kg N ha^{-1}. This difference is expected due to the difference in animal size between studies. Considering that average area allocated per day per group of animals was less in R (483 m^2) than in the other treatments (567 m^2) due to a higher herbage mass in R, there would be greater N loading per ha, as animals grazing on smaller areas are at a higher stocking density. As N loading in the urine patch is a key determinant of nitrate leaching (Di & Cameron 2007), this result suggests that diverse pastures have potential to reduce nitrate leaching.

Chapter 6

CONCLUSION

The results demonstrate a role for the use of chicory and plantain at proportions of 25 and 50% in the diet with perennial ryegrass-white clover irrigated pasture as mitigation tools to reduce the environmental impact of 8-9 month-old dairy heifer rearing systems in autumn. Although the exact mechanisms contributing to the marked reduction in urinary N excretion are yet to be fully understood, the results show no disadvantage to LWG in feeding chicory or plantain at 25 and 50% proportions in the diet compared to high quality irrigated ryegrass pasture.

This area of research has the potential for expansion, with the current trial only being completed over 32 days in autumn with 8-9 month-old dairy heifers. This work could be continued throughout the year to include differences in ryegrass, chicory and plantain growth. Future study could include different stock classes and look at the effect during different stages of heifer development. Furthermore, research could be done to determine the specific mechanisms around differences in urination frequency and N excretion. This could include observing temporal changes in water intake and urination behaviour over 24 hours and in relation to grazing bouts.

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